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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/531,844	04/18/2005	Haris Jahic	100874-1P US	8121

44992 7590 04/07/2006

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EXAMINER	
BULL, CHRISTOPHER	
ART UNIT	**PAPER NUMBER**
1655	

DATE MAILED: 04/07/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/531,844	JAHIC ET AL.	
	Examiner	Art Unit	
	Christopher Bull	1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 February 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-8* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *18 April 2005* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *18 Apr 2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claims 1-8 are presented for examination on the merits.

Specification Objections

The disclosure is objected to because of the following informalities:

The application as filed has seven drawings and seven corresponding Legends (Brief Description of Figures). However, the disclosure refers to Figure 8 (page 2, line 19). Figure 8 (as described on page 2 of the Disclosure, line 19) should show an alignment of the *E. faecalis* MurD sequence with several orthologues - other MurD enzymes. Figure 8 is shown in the parent application, 0224997.7, but not in PCT/GB03/045492. This problem could be fixed either by adding Figure 8 (with Legend), or by deleting the reference to the Figure in the Disclosure. This figure appears to form the basis for Claims 2-3.

There are three separate Sequence Identifier problems, as follows.

A) Sequence Identifiers missing in claim 5 and disclosure;

B) Insertions of Xaa into the sequence of SEQ ID NO: 2;

C) Mutations in nine residues of SEQ ID NO: 2; and

A) The Sequence Identifier is missing for the sequence shown on page 3 of the disclosure. This sequence corresponds to (see also below) SEQ ID NO: 2 in the Sequence Listing, but each sequence in the disclosure must be labeled with its SEQ ID NO: (see MPEP 2422 or 37 CFR 1.821 (d)). For Figures that includes sequences, a

SEQ ID NO: must be present for each sequence either in the Figure or in the corresponding Brief Description of the Figure (see MPEP 2422.02).

B) In SEQ ID NO: 2, there are seven extra residues of Xaa inserted at various locations in the Sequence Listing and in the Computer Readable Form (CRF), as well as SEQ ID NO: 1 and 3-4. In all four Sequence Listings, each Xaa is defined (by the "misc_feature" field) as being any naturally occurring amino acid. Therefore there is an amino acid residue at each Xaa position that must be counted in numbering the residue locations, as is shown in Sequence Listing. Perhaps because these Xaa residues were added in the course of aligning the sequences of SEQ ID NO: 1-4 to make (missing) Figure 8, Applicants have not counted these Xaa residues in determining the positions of the amino acid substitutions discussed on page 2 or in Claims 2 and 3. Whatever the cause, the residue numbering between SEQ ID NO: 2 and the amino acid substitutions of Claims 2-3 does not match.

An obvious way to remedy this problem is to delete the Xaa residues in SEQ ID NO: 1-4. This will require submission of: a substitute Sequence Listing, two new CRF disks, a Statement of identity between sequences on listing and disk, and a Statement that no new matter was introduced (follow the amendment procedure in 37 CFR 1.825 or MPEP 2426). This resubmission is also required to fix mutation problem C) below.

Note that if Figure 8 is returned to the application (having support in PCT/GB03/045492), it should remain aligned, with appropriate dashes, as that method of displaying an alignment is understood in the art. It is desirable to put contiguous sequences into SEQ ID NO: 1-4 but to leave alignment gaps such as in Figure 8.

Alignment gaps are not equivalent to a stretch of unknown or missing sequence information (the "gap" referred to at the end of MPEP 2423 about 37 CFR 1.822 (e)). For guidance, see MPEP 2423.03 (lower left of page 2400-40 Aug 01 version MPEP).

C) Even without the Xaa residues, the amino acid sequence in SEQ ID NO: 2 differs from the sequences listed on page 3 and in Claim 5, which are presumed correct. Omitting Xaa residues, SEQ ID NO: 2 contains the following nine mutations: P83L; V218A; A292P; V300A; R303K; T305S; I336F; E385K; and A394P. These mutations appeared in the *E. faecalis* sequence shown in Figure 8 of original submission to the EPO, continued into the Sequence Listing of the PCT, multiplied after publication, and are now found in new applications. If Figure 8 returns, all sequences must be correct.

Appropriate correction is required for the drawing/disclosure discrepancy and the three sequence issues.

Claim Objections

Claim 5 is objected to because it sets forth a sequence but has no Sequence Identifier. A Sequence Identifier must be included in any claim to a sequence, and may substitute for the sequence in the claim (see MPEP 2422.03 or 37 CFR 1.821 (d)).

Appropriate correction is required.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-5 and 7 are rejected under 35 U.S.C. 102(b) as being anticipated by Walsh et al. (1991 J Bact 181, 5395-5401, hereinafter Walsh), as evidenced by Pucci et al. (1997 J Bact 179, 5632-5635).

Walsh teach purifying and characterizing the MurD enzyme from four organisms (abstract page 5395). The MurD activity of the two gram-negative organisms, *Escherichia coli* and *Haemophilus influenzae*, depended strongly on the presence of ammonium or potassium ions as activators, while the two gram-positive organisms, *Staphylococcus aureus* and *Enterococcus faecalis*, were activator-independent (page 5400 upper left). Assays (page 5396) were run at 24°C in 100 mM tris/Cl buffer pH 8.0 by three methods: one fixed-time assay based on ^{14}C-labelled D-glutamate monitored by release of two products (HPLC separation with detection of both ADP by UV absorption and ^{14}C-labelled product by in-line scintillation); another fixed-time assay based on phosphate release from ATP (monitored in 96-well plates by the malachite green-molybdate reaction); and a continuous assay for phosphate release (monitored at 340 nm in a coupled assay to NADH). The affinity of each enzyme for its substrates and for a phosphinate mechanism-based inhibitor was measured using the latter method (except that *E. coli* used the radiolabel assay, page 5396). Walsh further teach contacting the enzyme with a test compound (i.e., the phosphinate inhibitor) in the presence of its substrates (reduced to near their Km values - page 5396, end of Assay

section) and appropriate buffers (Assay section again) and detecting a modulation of enzyme activity (the measured inhibition constant was 6 μM - page 5398 lower left).

Claim 1 recites "A method of identifying inhibitors of an activator-independent MurD enzyme comprising contacting the enzyme with a test compound in the presence of enzyme substrates and appropriate buffers and detecting any modulation of enzyme activity by the test compound." Walsh teach (page 5397 near end of section ii) that neither *Enterococcus faecalis* and *Staphylococcus aureus* MurD are activator-dependent, defined there as a six to twenty-fold activation by ammonium or potassium ions. Applicants have demonstrated that the MurD enzyme from *Staphylococcus aureus* shows an ammonium and potassium salt dependence (at two fold, notably smaller than that shown by Walsh for the two gram-negative organisms), but doing so for *Staphylococcus aureus* does not invalidate the teachings of Walsh with regard to the *Enterococcus faecalis* MurD. Thus, Walsh report an activator-independent MurD and detect modulation by an inhibitor, so the reference reads on all limitations of Claim 1.

The relation of Walsh to Claims 2-5 will be discussed in reverse order, because the flow of logic seems easier to grasp that way.

Claim 5 recites the method wherein the activator-independent MurD enzyme is an *Enterococcus faecalis* enzyme which has the sequence set forth in Claim 5. That sequence is the *Enterococcus faecalis* MurD enzyme sequence of Pucci et al. (1997 , recorded in the Swiss-Prot database 15 July 1998), which is cited here only to show the inherency of the *Enterococcus faecalis* MurD enzyme sequence that is the sequence of

Claim 5. Since Walsh also use the *Enterococcus faecalis* MurD enzyme, which necessarily has that same sequence, Walsh read on Claim 5.

Claim 4 recites the method wherein the activator-independent MurD enzyme is an *Enterococcus faecalis* MurD enzyme. Since Walsh use the *Enterococcus faecalis* MurD enzyme, Walsh read on Claim 5.

Claim 3 recites a set of amino acids at the indicated positions (listed in the *Enterococcus faecalis* MurD enzyme sequence using Applicant's numbering) that are the same amino acids that are present in *Enterococcus faecalis* MurD enzyme and are indicated in the specification to result in activator-independence. Since Walsh also used the same *Enterococcus faecalis* MurD enzyme, the enzyme taught by the reference reads on every residue location listed in Claim 3.

Claim 2 recites a set of amino acids at locations in a putative consensus sequence for three activator-dependent MurD enzymes (listed at the indicated positions in the *Enterococcus faecalis* MurD enzyme sequence using Applicant's numbering), one or more of which must not be in the claimed enzyme. Since Walsh also used the *Enterococcus faecalis* MurD enzyme, none of the listed amino acids were present in the MurD used by Walsh, so the reference reads on every location listed in Claim 2.

Claim 7 recites that the substrates be UDP-MurNac-L-Ala, D-glutamate and ATP. Since Walsh also used these substrates with the *Enterococcus faecalis* MurD enzyme, Walsh read on Claim 7.

Accordingly, the cited reference is deemed to anticipate Claims 1-5 and 7.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-5 and 7-8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Walsh in view of Tanner et al. (1996 J Org Chem 61, 1756-1760, hereinafter Tanner).

The teachings of Walsh have been discussed above and are applied as before to Claims 1-5 and 7. Additionally, Walsh beneficially state (page 5395 left col):

"... significant differences in cell wall structure exist between gram-positive and gram-negative bacteria. A better understanding on how bacteria coordinate and regulate the composition of these basic units may lead to development of more effective strategies to treat bacterial infections."

Walsh do not teach preincubating the enzyme with the test compound before adding substrates (as recited by Claim 8).

Tanner teach synthesis of active site analogs and testing on MurD from *E. coli*, (page 1758). Their best phosphinate based inhibitor had an IC_{50} below 1 μM (Compound 3, page 1758), and was tested both directly and after 10 minute preincubation with ATP and enzyme (page 1760). Tanner beneficially explain (page 1756 last paragraph) why preincubation with ATP and inhibitor is important for enzymes with mechanisms similar to MurD (such as MurF, the D-Ala-D-Ala ligase).

"In several cases it has been found that appropriately substituted phosphinic acids act as slow binding inactivators of these enzymes. The remarkable feature of this inhibition is that the enzyme promotes the transfer of the γ-phosphate of ATP onto the phosphinate anion to produce ADP and a phosphorylated inhibitor. ... The resulting phosphoryl phosphinate moiety closely mimics the tetrahedral intermediate formed in the normal reaction pathway and is tightly bound to the enzyme. In the case of the D-Ala-D-Ala ligase, an enzyme-inhibitor decomplexation half-life of 17 days was measured, which renders this noncovalent inhibition effectively irreversible."

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to employ a preincubation of inhibitor with ATP and enzyme when performing inhibitor studies on an *Enterococcus faecalis* MurD enzyme via the instantly claimed steps, for the following reasons. Walsh teach that it is within the ordinary skill in the art to clone an *Enterococcus faecalis* MurD enzyme and search for inhibitors of this enzyme as possible antibacterial agents. Tanner teach that it is within the ordinary skill in the art to design inhibitors that may require preincubation with enzyme and ATP to exert full inhibitory potential.

One would have been motivated to combine these teachings for the expected benefit of finding better antimicrobial agents for gram positive bacteria, based on the rational design of inhibitors of the MurD enzyme, as taught by both references.

Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claims 1-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Walsh in view of El-Sherbeini et al. (1998 Gene 210, 117-125 hereinafter El-Sherbeini) and Bouhss et al. (1999 Biochem 38, 12240-12247, hereinafter Bouhss).

The teachings of Walsh have been discussed above and are applied as before to Claims 1-5 and 7. Additionally, Walsh beneficially state (page 5395 left col) that:

"... significant differences in cell wall structure exist between gram-positive and gram-negative bacteria. A better understanding on how bacteria coordinate and regulate the composition of these basic units may lead to development of more effective strategies to treat bacterial infections."

Walsh do not teach shortening an *Enterococcus faecalis* MurD enzyme by up to 16 residues from the N-terminus or by up to 12 residues from the C-terminus (as recited by Claim 8).

El-Sherbeini teach a comparison of the structure of the cell wall synthesis gene clusters, showing a particularly similar relationship between *Enterococcus faecalis, Enterococcus hirae* and *Streptococcus pyogenes* (page 120, Fig 1). They present a sequence alignment of five MurD enzymes including *Enterococcus faecalis* (Fig 3, page

122), which shows a region of conserved amino acids starting at G12 in the *E. coli* numbering and continuing for the next 6 residues for the four gram-positive sequences. That figure also shows a region of significant homology near the C-terminus, extending to F429 in the *E. coli* numbering. From Figure 3 showing the *Enterococcus faecalis* sequence, it is apparent that G12 and F425 correspond to G16 and F444 (in the *Enterococcus faecalis* numbering), placing these amino acids 16 residues in from the N-terminus and 12 residues in from the C-terminus. Additionally, El-Sherbeini beneficially state (mid left, page 118):

"... it is imperative to identify novel compounds that have a broad-spectrum antimicrobial activity. One approach to the discovery of such compounds is to screen for inhibitors of key biochemical targets (like MurD)."

El-Sherbeini further compare the activity of their MurD with another MurD (page 124 upper left) and beneficially state (lower left, page 124) the next step of determining:

"... whether these different enzymes have similar kinetic parameters or whether they will show the same sensitivity to known inhibitors of the MurD reaction, for example to a transition state analog ...."

Bouhss present a sequence alignment of 26 MurD enzymes including *Enterococcus faecalis* (Fig 1, page 12243). It shows that G12 (G16 in the *Enterococcus faecalis* numbering) and F429 (F444 in the *Enterococcus faecalis* numbering) are conserved residues in all 26 MurD sequences. The regions between the N-terminus and G12 and between the C-terminus back to F429 do not show significant homology. Bouhss also show (page 12244, Fig 2) the crystal structure of the MurD, from which it is clear that the N-terminal region before G12 and the C-terminal region after F429 both

extend away from the active site and lie on the exterior of the protein. Additionally, Bouhss beneficially investigate the effect of 14 alterations of active site amino acid residues by assaying for their activity and further state (end of page 12240):

"... the Mur synthetases represent potential targets for new antibacterial compounds. Therefore, the identification of amino acid residues involved in the catalytic mechanism of in the binding of substrates could lead to the rational design of inhibitors."

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made that residues as conserved as G16 and F444 must play important roles in the activity of MurD, whereas portions of the N and C terminal tails which are not so conserved nor near the active site could be deleted without probable loss of function. It would also have been obvious to one of ordinary skill in the art at the time the claimed invention was made that such constructs (i.e., deletions from the N-terminal 15 residues in to Gly 16 and from the C-terminal 12 residues back to F444) were as easily prepared as the mutated residues then being tested. It would further have been obvious to one of ordinary skill in the art at the time the claimed invention was made to assay these shortened MurD polypeptides as part of a method of performing inhibitor studies on an *Enterococcus faecalis* MurD enzyme via the instantly claimed steps, for the following reasons. Walsh teach that it is within the ordinary skill in the art to perform genetic engineering on an *Enterococcus faecalis* MurD enzyme and to assay mutants of this enzyme as part of a search for inhibitors as possible antibacterial agents. El-Sherbeini teach that it is within the ordinary skill in the art to ascertain conserved regions of sequence within MurD, imperative to find inhibitors of this enzyme, and assay to learn about the active site activity. Bouhss teach that it is

within the ordinary skill in the art to design inhibitors of MurD by altering portions of the enzyme and assaying these mutants to locate the functional limits of the MurD active site, so as to aid in the design of inhibitors of that enzyme.

One would have been motivated to combine these teachings for the expected benefit of finding better antimicrobial agents, based on the rational design of inhibitors of the MurD enzyme, as taught by all three references.

Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Conclusion

No claims are allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

El-Sherbeini et al. patent (US 6,746,858 issued June 2004 but filed 3 May 2000) is available as 102(e) art.

Dementin et al. (Nov 2001 Eur. J. Biochem. 268, 5800-5807) shows chemical rescue of mutants at K198 in the MurD from *E. coli*, as well as significant salt activation by Mg^{++} which is not excluded by applicant's definition of activator-independent and likely true for the *Enterococcus faecalis* MurD enzyme.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Christopher Bull whose telephone number is (571) 272-1327. The examiner can normally be reached on 7:30-4.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey can be reached on (571) 272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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cb

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